



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :  A61K 47/00		A1	(11) International Publication Number: <b>WO 00/09163</b>
			(43) International Publication Date: 24 February 2000 (24.02.00)
<p>(21) International Application Number: PCT/US99/18094</p> <p>(22) International Filing Date: 10 August 1999 (10.08.99)</p> <p>(30) Priority Data: 09/132,593 11 August 1998 (11.08.98) US</p> <p>(71) Applicant (for all designated States except US): CONLINCO, INC. [US/US]; Suite 308, 211 W. Holmes Street, Detroit Lakes, MN 56501 (US).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only): SAEBO, Asgeir [NO/NO]; Industriverin, N-6160 Hovdebygda (NO). SKARIE, Carl [US/US]; Suite 308, 211 W. Holmes Street, Detroit Lakes, MN 56502 (US).</p> <p>(74) Agents: JONES, J., Mitchell et al.; Medlen &amp; Carroll, LLP, Suite 2200, 220 Montgomery Street, San Francisco, CA 94104 (US).</p>			<p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published With international search report.</p>
<p>(54) Title: CONJUGATED LINOLEIC ACID ALKYL ESTERS IN FEEDSTUFFS AND FOOD</p> <p>(57) Abstract</p> <p>A novel method of delivering conjugated linoleic acid to an animal involves compounding feed or food for human consumption with a biologically active amount of conjugated linoleic acid alkyl ester (CLA-ester). The CLA-ester comprises a mixture of c9,t11-octadecanoic acid and t10,c12-octadecanoic acid, with contaminating isomers being present at an aggregate percentage of less than 5. Manufactured from sunflower or safflower oil, the CLA-esters contain less than 0.5 percent phosphatidyl residue, so that a food grade product is made without special purification and refining steps.</p>			

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		

## CONJUGATED LINOLEIC ACID ALKYL ESTERS IN FEEDSTUFFS AND FOOD

### 5 FIELD OF THE INVENTION

This invention relates to the field of nutrition and the supplementation of feedstuffs and food with alkyl esters of conjugated linoleic acid. A process for making the esters utilizes a nonaqueous alcoholate catalyzed reaction to convert the alkyl esters of linoleic acid derived from sunflower and safflower oil to predominately the c9,t11- and t10,c12- conjugated isomers.

### BACKGROUND OF THE INVENTION

The biological activity of conjugated linoleic acids (hereinafter CLA) has been well documented in a number of indications. Its effect as an anticarcinogenic agent was demonstrated in a rat mammary tumor model by Ha, et al., *Cancer Res.*, 52: 2035s (1992), and in a mouse forestomach neoplasia model (Ha, et al., *Cancer Res.*, 50: 1097 (1990). CLA has been found effective in attenuating allergic reactions mediated by type I or TgE hypersensitivity. As a nutritive supplement, CLA administration results in selective reduction in body fat, as disclosed in U.S. Patent No. 5,554,646, and has a significant positive effect on feed conversion efficient as shown in U.S. Patent No. 5,428,072.

Linoleic acid is an important component of biolipids, and comprises a significant proportion of triglycerides and phospholipids. It is an essential fatty acid, in that it is required in the diet for maintenance of healthy cells, but the body does not possess the enzymatic machinery to synthesis the fatty acid itself. Linoleic acid has 18 carbon atoms with double bonds at positions 9 and 12. The conjugated forms of linoleic acid have the double bond positions shifted so that the double bond pairs are separated by a single methylene group. The rearrangement of the double bonds of linoleic acid to conjugated positions results in eight possible geometric isomers of 9,11 and 10,12 octadecadienoic acid (c9,c11; c9,t11; t9,c11; t9,t11; c10,c12; c10,t12; t10,c12; and t10,t12. Other minor conjugated forms appear in nature and result from synthetic processes, namely, ct8,ct10 and ct11,ct13 being the most prevalent.

A general mechanism for the isomerization of linoleic acid was described by J.C. Cowan in JAOCS, 72: 492 (1950). It is believed that the double bond is polarized by the result of a collision with an activating catalyst. The polarized carbon atom and its adjoining carbon are then free to rotate and the forces are such to make the deficient carbon atom essentially planar. When the system relieves forces set up as a result of the molecular collision, both cis and trans isomers are formed. More of the 10,12 and 9,11 isomers are formed than other species because of the thermodynamic stability of these forms. More severe conditions of heat, pressure, and polarity tend to drive isomerization further to the more stable trans,trans isomers, and cause redistribution of the double bonds with the appearance of significant quantities of the 8,10 and 11,13 forms.

One problem with aqueous alkali isomerization, which is the principal industrial process for producing CLA, is the formation of these multiple species. The reaction becomes uncontrolled and a significant proportion of the linoleic acid substrate is sacrificed to undesirable trans, trans isomers. For industrial use in drying oils where generalized polymerization between fatty acid strands is sought, it makes little difference which species of conjugated isomer predominate. However, in therapeutic or nutritional applications, the t10,c12 and c9,t11 isomers are believed to contain most, if not all, of the biological activity.

Other methods have been described utilizing metal catalysts, which are not highly efficient. Isomerization in these systems could be achieved more rapidly in the presence of higher molecular weight solvents. Kass, et al., J. Am. Chem. Soc., 61: 4829 (1939) showed that replacement of ethanol with ethylene glycol resulted in both an increase in conjugation in less time. U.S. Patent No. 2,350,583 and British Patent No. 558,881 (1944) achieved conjugation by reacting fatty acid soaps of an oil with an excess of aqueous alkali at 200-230 degrees C. and increased pressure. Among the processes known to cause isomerization in the absence of aqueous alkali, is a nickel-carbon catalytic method, as described in Radlove, et al., Ind. Eng. Chem., 38: 997 (1946).

Processes have also been described for isomerization of polyethanoid fatty acids in their ester forms. U.S. Patent Nos. 2,242,230 and 3,162,658 disclose methods in which the lower alkyl esters of linoleic acid are isomerized by catalysis with basic

alcoholates, preferably sodium or potassium at moderate temperatures in the range of 100-140 degrees C. Typically these processes are used to generate industrial drying oils, and hence predominately utilize soy and corn oil as the starting material, in order to enhance polymerization when coated onto surfaces. These fatty acid ester compositions are not suitable for human or animal consumption because of high phosphidyl and other residue content. Purification by distillation, differential extraction, and the like removes the residues, but also causes further double bond rearrangements giving an unacceptable level of trans,trans CLA isomers, and intermolecular polymers.

The purified CLA utilized in prior feeding studies was obtained by small scale laboratory procedures involving production of CLA from highly purified linoleic acid. For example, Sullivan, J. Am. Oil Chemists' Soc., 53: 359 (1976) describes a laboratory semi-pilot steam refining system made entirely of glass. While such systems are adequate for producing quantities of CLA for laboratory studies, or even clinical trials, they are not suitable for commercial scale bulk production. On the other hand, the large scale systems available to produce industrial quantities of CLA cannot be run inexpensively enough to produce material for bulk animal feeds. The degumming, refining, and dehydration steps necessary to obtain nutritionally safe edible CLA for livestock feeding are prohibitively complex and expensive.

Economical CLA-ester production in commercial quantities is a desirable objective in light of nutritional benefits observed on a laboratory scale. The advantages of an ester derivative rather than the free CLA fatty acids include resistance to oxidation, ease of manufacture according to the process of the present invention, palatability, and compatibility with lipid feed components.

## 25 SUMMARY OF THE INVENTION

In the present invention, a feed safe conjugated linoleic acid alkyl ester is manufactured under conditions preferentially controlling isomerization to the desired 10,12 and 9,11 isomers, while limiting formation of 8,10; 11,13; and trans,trans species. Such conditions are met by employing an alkali alcoholate catalyzed reaction in which a seed oil is split to release free fatty acids from a glycerol backbone and then esterifying prior to isomerization. The key to an adaptation of this process to a commercially viable product is reduction in the process steps which add cost.

Typically, residues derived from non-oil components of seed oils, such as sterols and phosphatides, foul equipment and reduce palatability for feed or food use. In the case of typical seed oils such as soy or corn these residues are present in sufficient quantity that a CLA-ester product could not be used in consumable products.

5 In the composition of the present invention, non-oil residues are not purified away from the oil component, but rather the source of oil is selected to maintain such residues at acceptable levels. By selecting safflower or sunflower oil as an oil source, critical residue levels can be controlled to between 0.1 and 0.5% phosphatides, and to an unsaponifiable sterol fraction containing between 5 and less than 20 percent each of 10 campesterol and stigmasterol, without extensive degumming and distillation processing steps. The resulting linoleic acid alkyl ester is comprised of at least 50 percent up to about 99 percent by weight of octadecadienoic acid ester isomers representing combinations of various possible individual percentages of c9,t11-octadecadienoic acid alkyl ester and t10,c12-octadecadienoic acid alkyl ester. In the alkali alcoholate 15 catalyzed process roughly equal amounts of each of these ester isomers are produced, but the relative percentages can be altered by addition of one or the other of a composition enriched for one isomer. The CLA ester may then be incorporated into an animal feed by compounding the feed from conventional ingredients in a ration typical for the species and age of the animal, and blending therewith the conjugated linoleic 20 acid alkyl esters in a biologically active concentration, generally about 0.05 to 3.5 percent by weight.

25 The CLA-ester product of the present invention is obtained by direct isomerization of an unrefined linoleic acid, e.g. a linoleic acid source not subjected to refining steps. The CLA-ester composition has one part comprising at least 50 percent by weight of ester isomers (up to substantially 100 percent) of a mixture of ester isomers of c9,t11-octadecadienoic acid ester and t10,c12 -octadecadienoic acid ester, a second part comprising less than about 10 percent, preferably less than about 1 percent by aggregate weight of ester isomers of the structure 8,10-octadecadienoic acid ester, 11,13-octadecadienoic acid ester, and trans,trans-octadecadienoic acid esters, and a third 30 part containing a phosphatidyl residue of between 0.1 and 0.5 percent of the total composition weight. The alkyl groups may be methyl, ethyl, propyl, isopropyl, butyl, isobutyl and the like. Adjustments in concentration of the c9,t11 and t10,c12 isomers

5

can be made by addition of a composition enriched for one or the other isomer to yield an ester composition wherein the c9,t11, or the t10,c12 respectively contained in the first composition part constitutes greater than 60 percent, preferably greater than about 66 percent, most preferably greater than about 92 percent of the total isomers of octadecadienoic acid esters.

10

In the process embodiment of the present invention resulting in a food grade composition suitable for an animal feed, food ingredient, or human dietary supplement, an unrefined CLA-ester having a phosphatidyl residue less than 0.5 percent is treated with an alkali alcoholate in the presence of a monohydric low molecular weight alcohol such as methyl or ethyl alcohol, continuing the treatment at low temperature (about 90 to 145 degrees C.) until at least 50 percent of the ester is converted to CLA-ester, acidifying by addition of an aqueous acid, and then separating the CLA-ester from the aqueous acid without a distillation step.

15

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 is a flow diagram of the present process, in which esterification is followed directly by isomerization without a purification step.

20

Figure 2 is a rectilinear plot showing the relation between aqueous alkali processing temperature and the yield of the 9,11 and 10,12 isomers compared to total conjugated linoleic acids.

#### **DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS**

25

Various methods of producing conjugated double bonds by alkali isomerization are known in the art. U.S. Patent 2,350,583 (Bradley, 1944) describes a method of producing conjugated fatty acids by aqueous alkali isomerization. This method resulted in the conjugation of about 50% of the double bonds present in the polyunsaturated fatty acids used. U.S. Patent 2,242,230 (Burr et al., 1941) describes a method of non-aqueous alkali conjugation of fatty acids, resulting in the conjugation of approximately 100 percent of the double bonds in the polyunsaturated fatty acids studied. Another process resulting in the efficient formation of conjugated double bonds is described in U.S. Patent 4,381,264 (Struve, 1983). There, the inventors treat polyunsaturated fatty

5

acids with  $\text{SC}_2$  in the presence of substoichiometric amounts of soap forming bases. Perhaps the most commercially viable method for producing large quantities of conjugated fatty acids is the continuous flow aqueous alkali isomerization process described in U.S. Patent 4,164,505. This process results in essentially all available double bonds being conjugated in a short reaction time. The foregoing patents are incorporated herein by reference.

10

15

In the production of a food grade CLA-ester, a laboratory scale procedure utilizes reagent grade 9,12-linoleic acid alkyl ester in a reaction as disclosed in U.S. Patent Nos. 2,242,230 and 3,162,658, hereby incorporated by reference. The reaction is carried out at about 100-140 degrees C. in a closed vessel for 5-8 hours. Isomerization is substantially complete. The resulting product is perfectly safe for human or animal consumption. However, the use of reagent grade linoleic acid esters is impractical for compounding into bulk feeds, or even for human consumption in capsule form. On the other hand mere isomerization of the linoleic acid contained in a crude or unrefined linoleic ester, as is suitable for production of the industrial drying oils contemplated by the above cited patents, will not yield a food grade because of the high phosphatidyl and sterol content, as well as a tendency for oils containing high levels of these impurities to polymerize.

20

Applicants have discovered that if sunflower or safflower oil is utilized, the resulting phosphatidyl and sterol residues are low enough for the material to be characterized as food grade. In particular, Applicants first provide the sunflower or safflower as delivered by tanker, subject it to fat slitting and esterification, and then proceed directly with the unrefined esterification product into isomerization. The usual purification and refining steps are not necessary. This material can be made in

25 commercial scale quantities at a low enough cost to be practical as a feed or food supplement.

25

Referring now to the flow diagram of Figure 1, after fat splitting and dehydration, the free fatty acids are combined with methanol or other monohydric low molecular weight alcohol and heated to the temperature at which the alcohol boils. Esterification proceeds under refluxing conditions with removal of the reaction water through a condenser. After the addition of a further quantity of the same or a different monohydric alcohol an alcoholate catalyst is blended into the ester mix. Typical

alcoholate catalysts are sodium or potassium ethoxide, or their methyl, butyl, or propyl counterparts.

In the esterification, methanol or ethanol are preferred, although other branched or straight chain monohydric alcohols may be used. The longer the aliphatic chain of the alkyl group, the more lipid compatible the material becomes. Also the viscosity tends to increase. For different types of feed or food, whose consistency varies, product of varying viscosity can be used to obtain the desired flow or compounding characteristics without affecting the therapeutic or nutritional properties arising from the CLA moieties. The theory and practice of esterification are conventional. A basic explanation of the most common methods is set forth in the McCraw-Hill Encyclopedia of Science & Technology, McGraw-Hill Book Co., N.Y.: 1996 (5th ed.). The animal and human body has a variety of esterases, so that the CLA-ester is cleaved to release the free fatty acids readily. Tissue uptake may have a different kinetics depending on the tissue involved and the benefit sought.

In the isomerization step, it was found that alcoholate catalysis produced a much superior product than aqueous alkali mediated isomerization. The latter process always produced undesirable isomers even under mild reaction conditions. The milder conditions do give lower amounts of unwanted isomers, but at the great expense of yield, as shown in the Examples. In most systems the appearance of the c9,t11 and t10,c12 isomers dominates and they are formed in roughly equimolar amounts. It has not heretofore been possible to control the isomerization of the one isomer to the exclusion of the other. While it is desirable to increase the percentage of one or the other isomer (depending on the physiological effect to be achieved), at present this must largely be carried out by adding an enriched source of the desired isomer.

Crude sunflower or safflower oil is the preferred fatty acid source for producing CLA. Sunflower oil contains a high amount of linoleic acid (about 65% on average). Safflower oil typically contains even higher amounts (greater than 70%). Preferably, a hexane extract of crude, non-degummed oil is the starting substrate for CFAP production. This extract is commercially available and is the same quality as the oil used as the starting point for edible products. The ability to use raw sunflower or safflower oil as the starting substrate provides an important economic advantage because it is less expensive than refined sunflower oil.

Applicants have discovered that other raw oils, such as raw corn and soybean oils, are not suited to the present new use of CLA in bulk feeds because of the production of polymerized products during the fat splitting and conjugation processes, and because of the high phosphatide content. Also, certain sterols such as campesterol and stigmasterol are known to have a tendency to foul processing equipment during conjugation and plug nozzles during materials transfer. The polymerization by-products also result in loss of yield from these other oils, even though at first glance the other oils may seem to have more desirable properties. Corn oil (about 56% linoleic acid) and soybean oil (about 50-55% linoleic acid) have comparable linoleic acid contents as compared to sunflower oil (about 60% linoleic acid). These oils are inexpensive and large quantities are available, which make them attractive candidates as a potential source of CLA for bulk feeds. However, their use for commercial CLA production is substantially lower per unit quantity of oil because the ultimate yields of CLA are lower than for sunflower or safflower oil, and because of the added expense for additional 10 cleaning and purification steps.

Heat sensitive triglycerides containing multiple double bonds are abundant in oils having an iodine value above 120. Oils containing such heat-sensitive triglycerides have a tendency to form polymers when subjected to continuous countercurrent fat-splitting. These polymers become insoluble in oil and will foul equipment, resulting in lowered efficiencies of splitting and yields. Sunflower oils are classified as heat-sensitive because of their high linoleic acid content and iodine number. Sunflower oil has an iodine number of 130; safflower oil of about 145. Soybean oil has an iodine number of about 132, and corn oil has an iodine number of about 130. All these oils have high linoleic acid contents and iodine numbers above 120, thus belonging to the heat-sensitive group. It is therefore surprising that sunflower and especially safflower oils can be split and conjugated by commercial processes with few processing complications.

The prevalent phosphatides are phosphatidylethanolamine, phosphatidylinositol and phosphatidylcholine. The phosphatide content of sunflower and safflower oils is about 0.4 to 1.0% as reported in Klein and Crauer, JAOCs 51:382A-385A and Burkhardt, JAOCs 48:697-699 (1971), respectively. In contrast, the phosphatide content of soybean oil is about 1.5-2.5% as reported in the Handbook of Soy Oil

Processing and Utilization, Erickson et al. eds., AOCS, Champaign, 1980. For crude oils with low amounts of these substances, such as sunflower and safflower oils, proceeding can advance directly to esterification and isomerization after degumming.

5

### EXAMPLE 1

#### **Effect of Varying Temperature and Reaction Duration on CLA Yield and Composition**

The effect of temperature and reaction duration on the conjugation of safflower oil was determined. Water and NaOH were added to a high pressure reactor (Parr Model 450 ML Benchtop Alloy 400, equipped with a pressure gauge and stirrer) as indicated in Table 1, columns 1 and 2. The NaOH was allowed to dissolve and safflower oil (column 3) was added to the reactor. The reactor was closed and flushed for 2 min. with nitrogen and then all valves were closed. The reactor was heated in an electrical gasket to the desired temperature (column 4) and maintained at that temperature for the desired time (column 5). The temperature was then reduced to 60°C before pressure was released and the reactor opened. For each reaction, two grams of the resulting solidified soap were taken from the reactor and dissolved in water at approximately 40°C. Citric acid was then added to reduce the pH of the solution to below 6. A sample was withdrawn from the fatty acid top layer and prepared for Gas Chromatography.

The results of the gas chromatography are presented in column 6 (total percentage of 9,11 and 10,12 isomers), column 7 (total percentage of 11,13 isomers), and column 8 (total percentage of all CLA isomers or yield). These data indicate that as reaction duration and temperature increase, the total amount of conjugation and the percentage of 11,13 isomers increase. Under conditions where formation of the 11,13 isomer is low, the total amount of conjugation is also low.

Figure 2 further illustrates the above findings. As temperature is increased, total CLA also increases, but the proportion of the 9,11 and 10,12 isomers declines. At temperatures above 189°C, the absolute amounts of these isomers also declines. The CLA species favored under these conditions are the trans,trans isomers.

**TABLE 1: Conjugation in Water Solvent  
Summary of Results**

Water gram	NaOH gram	Safflower Oil gram	Mean t. °C of reaction	Time hours	9,11+ 10,12 area%	11,13 area%	CLA total area%
50.21	29.93	99.94	189	6.36	45.99	5.73	55.86
70.20	29.93	99.94	187	6.40	44.94	3.23	51.28
50.10	30.17	100.74	183	6.39	40.23	3.37	48.07
49.91	29.93	100.40	179	6.52	32.00	1.48	34.92
49.97	29.80	100.02	179	10.08	41.86	3.12	48.21
49.94	39.84	99.84	179	6.30	32.6	3.04	37.12
29.50	24.83	99.21	240	3.25	28.37	10.78	71.58
30.33	25.15	100.43	221	2.30	40.87	14.72	72.61
49.92	30.00	100.36	150	6.34	7.07	0	7.44

### EXAMPLE 2

### Conjugation of Safflower Fatty Acid Methyleneester (FAME)

The reaction was carried out in a closed vessel. The following components were mixed together: 100g safflower FAME and a mixture of approximately 2.8 g KOCH<sub>3</sub> and 2.8 g methanol. There was probably more KOMe than methanol due to evaporation of methanol during mixing of the two components. The mixture was stirred for 5 hours at 111-115 deg C in nitrogen atmosphere in a closed reaction vessel. The distribution of isomers was analyzed by Gas Chromatography. The results are summarized in Table 2. The raw GC data is presented in Table 3. These data indicate that the conjugation safflower FAME may be accomplished under mild conditions, resulting in a product lacking appreciable amounts of undesirable 8,10 and 11,13 isomers.

TABLE 2: Isomer Distribution

	Palmitic acid	6.6%
	Stearic acid	2.7%
5	Oleic acid	12.9%
	Linoleic acid	5.7% (unconjugated)
	CLA c9,t11	34.1%
	CLA t10,c12	33.3%
10	CLA c,c	1.8%
	CLA t,t	1.0%
	CLA total	70.2%

EXAMPLE 315      **Large Scale Batch Production of Conjugated Safflower FAME**

The production of safflower conjugated FAME may be divided into two steps, methanolysis and conjugation. For methanolysis, 6,000 kg safflower oil was drawn into a closed reactor. The reactor was purged with nitrogen at atmospheric pressure, and 20 1150 liters of methanol and 160 kg of NaOCH<sub>3</sub> (30% solution) were added. The mixture is heated to 65°C while stirring, and reacted at 65°C for 2 hours. The resulting bottom layer was decanted while the reactor was purged with nitrogen gas. 1000 liters of water (40-50°C, into which 50 kg citric acid monohydrate has been dissolved) was then added while stirring. The layers were allowed to separate (approx. 60 min.) and 25 the bottom layer decanted while purging the reactor with nitrogen gas. The resulting safflower FAME product was dried at 80°C under vacuum for one hour.

To conjugate the safflower FAME, 250 kg of KOCH<sub>3</sub> dissolved in methanol to form a paste was added to the reactor. The mixture was then heated to 120°C while stirring and the reaction allowed to continue for 3 hours. The mixture was cooled to 30 100°C, and 1000 liters of water (40-50°C, into which 50 kg citric acid monohydrate has been dissolved) was added while stirring. The mixture was stirred for 15 minutes and

then the layers were allowed to separate for 20 minutes. The bottom layer was decanted and the product dried at 80°C for 1 hour and then stored under nitrogen.

The resulting CLA was analyzed using a Perkin Elmer Autosystem XL GC under the following conditions:

5           Column:    WCOT Fused Silica 100 m X 0.25 mm, Coating CP-SIL 88  
Carrier:    He gas, 30.0 PSI  
Temp:       220 C  
Run time:   35-90 min.  
Inject.:     Splitless, 240 C  
10           Detect.:   FID, 280 C

The GC results are summarized in Table 3.

TABLE 3: Gas Chromatography Results

Peak #	Time (min)	Component Name	Area (%)	Area ( $\mu$ Vs)	Height ( $\mu$ V)
5	46.874	C16:0	6.37	29874.50	4026.29
	58.685	C18:0	2.61	12231.70	1542.34
	62.141	C18:1 c9	13.14	61668.78	7369.08
	62.652		0.70	3263.62	391.92
10	66.404		0.35	1627.60	177.41
	66.917		0.26	1239.15	157.35
	67.583	C18:2 c9,c12	5.75	26964.95	3153.80
	70.631		0.25	1171.90	141.41
15	75.011	CLA c9,t11	34.42	161529.90	17544.79
	75.936	CLA t10,c12	33.48	157129.82	17157.21
	76.400	CLA c9,c11	0.84	3935.70	302.61
	76.631	CLA c10,c12	0.49	2316.98	279.31
	77.905	CLA t, t 9,11+ 10,12	1.35	6344.50	710.88
			100.00	469299.10	52954.41

TABLE 3 cont.

Peak #	Time (min)	Component Name	Area (%)	Area ( $\mu$ Vs)	Height ( $\mu$ V)
9	75.011	CLA c9,t11	48.76	161529.90	17544.79
10	75.936	CLA t10,c12	47.43	157129.82	17157.21
11	76.400	CLA c9,c11	1.19	3935.70	302.61
12	76.631	CLA c10,c12	0.70	2316.98	279.31
13	77.905	CLA t,t 9,11+ 10,12	1.92	6344.50	710.88
			100.00	331256.90	35994.80

The following are examples of typical animal rations containing the CLA esters of the present invention.

15

#### EXAMPLE 4A

#### PIG STARTER RATIONS

20

Ingredients	lbs.	kgs.
Corn, yellow (8.4% protein)	1067	484.7
Soy bean meal, solvent extracted, dehulled (47% protein)	570	259
CLA-ester	5	2.3
Whey, dried (12.0% protein)	300	136
Dicalcium phosphate	24	11
Limestone	16	7
Iodized salt	5	2
Trace mineral premix	5	2
Vitamin premix	8	4
<b>Totals</b>	<b>2000</b>	<b>908</b>

EXAMPLE 4B**GROWER-FINISHER RATIONS FOR PIGS  
(FROM 40-240 LBS[18-109KGS])**

5

10

15

20

25

30

Ingredients	lbs.	kgs.
Corn, yellow (8.4% protein)	1566	
Soybean meal, solvent extracted (44% protein)	380	
CLA-ester	5	
Dicalcium phosphate	21	
Limestone	15	
Iodized Salt	5	
Trace Mineral Premix	3	
Vitamin Premix	3	
<b>Total</b>	<b>2000</b>	

EXAMPLE 4C**PIG GROWER FINISHER RATIONS  
(FOR PIGS 121-240LBS[55-109KGS])**

Ingredients	lbs.	kgs.
Corn, yellow (8.4% protein)	1687	
Soybean meal, solvent extracted (44% protein)	265	
CLA-ester	5	
Dicalcium phosphate	18	
Limestone	15	
Iodized salt	5	
Trace mineral premix	2	
Vitamin premix	3	
<b>Total</b>	<b>2000</b>	

EXAMPLE 4D**COMPOSITION AND ANALYSIS OF  
PIG TRACE MINERAL REMIX**

5

Element	Source	Amount (lbs)
Copper (Co)	Copper Sulfate	1.500
Iodine (I)	Potassium Iodide	0.010
Iron (Fe)	Ferrous Sulfate	25.000
Manganese (Mn)	Manganese Sulfate	2.500
Selenium (Se)	Sodium Selenite	0.025
Zinc (Zn)	Zinc Sulfate	25.000
	Carrier	45.965
<b>Total</b>		<b>100.000</b>

10

15

EXAMPLE 4E**COMPOSITION OF PIG VITAMIN PREMIX**

20

Vitamins	Amount
<b>Essential</b>	
Vitamin A.....(million IU)	5.0
Vitamin D.....(million IU)	0.6
Vitamin E.....(thousand IU)	26.0
Niacin.....(g)	25.0
d-Pantothenic acid....(g)	20.0
Riboflavin.....(g)	6.0
Vitamin B-12.....(mg)	25.0
<b>Optional</b>	
Biotin.....(g)	0.3
Menadione.....(g)	4.0
Carrier.....	to 10lbs
<b>Total</b>	<b>10.0</b>

35

EXAMPLE 4F

## 18% PROTEIN LAYER RATIONS FOR HENS

	Ingredients	lbs.	kgs.
5	Ground yellow corn	1242	564.5
	CLA-ester	5	2.3
10	Alfalfa meal, 17%	25	11.3
	Soybean meal, dehulled	451.6	205.3
15	Meat and bone meal (47%)	50	23.0
	DL-methionine	1.0	.5
20	Dicalcium phosphate	7	3.1
	Ground limestone	174	79.1
25	Iodized salt	7	3.1
	Stabilized yellow grease	37	17.2
	<b>Mineral and vitamin supplements</b>		
	Calcium pantothenate (mg)	5,000	
	Manganese (g)	52	
	Selenium (mg)	90.8	
	Zinc (g)	16	
	Vitamin A (IU)	6,000,000	
	Vitamin D <sub>3</sub> (IU)	2,000,000	
	Choline (mg)	274,000	
	Niacin (mg)	12,000	
	Riboflavin (mg)	2,000	
	Vitamin B-12	6	
	<b>Total</b>	<b>2000</b>	<b>909.4</b>

**EXAMPLE 4G**  
**STARTER AND FINISHER RATIOS FOR BROILERS**

Ingredients	Starter (up to 24 days)		Finisher (25 days to market)	
	lbs.	kgs.	lbs.	kgs.
Ground yellow corn	1,106	503	1235	561
CLA-ester	5	2.3	5	2.3
Soybean meal, dehulled	605	275	420	191
Alfalfa meal, 17%	-	-	25	11
Corn gluten meal, 60%	50	23	75	34
Fish meal, herring, 65%	50	23	50	23
Meat and bone meal, 47%	50	23	50	23
Dicalcium phosphate	10	4	9	4
Ground limestone	16	7	14	6.3
DL-methionine	0.8	0.3	-	-
Stabilized yellow grease	101	45.7	110	49.4
Iodized salt	7	3	7	3
<b>Mineral and vitamin supplement</b>				
Calcium pantothenate (mg)	5,000		5,000	
Manganese (g)	75		75	
Organic arsenical supplement	0.1		0.1	
Selenium (mg)	90.8		90.8	
Zinc (g)	30		30	
Vitamin A (IU)	4,000,000		4,000,000	
Vitamin D (IU)	1,000,000		1,000,000	
Vitamin E (mg)	2,000		2,000	
Vitamin K (mg)	2,000		2,000	
Choline (mg)	503,000		672,000	
Niacin (mg)	20,000		20,000	
Riboflavin (mg)	3,000		3,000	
Vitamin B-12 (mg)	12		12	
<b>Total</b>	<b>2000.9</b>	<b>909.3</b>	<b>2000.1</b>	<b>909.5</b>

EXAMPLE 4H

## GROWER/FINISHER TURKEY RATIONS

5

Ingredients	Grower (8-16 weeks)		Finisher (16 weeks-market)	
	lbs.	kgs.	lbs.	kgs.
Ground yellow corn	1194	595	1490	677.2
Wheat middlings	50	23	-	-
Alfalfa meal, 17%	25	11.3	25	11.3
Soybean meal, dehulled	570	259	335	152.3
Meat and bone meal, 47%	50	23	50	23
Dicalcium phosphate	32	14.5	23	10.5
Ground limestone	14	6	17	8
Stabilized yellow grease	45	20.7	45	20.7
CLA-ester	5	2.3	5	2.3
Iodized Salt	10	4.5	10	4.5
<b>Mineral and vitamin supplements</b>				
Calcium pantothenate (mg)	4,500		4,500	
Manganese (g)	30		30	
Selenium (mg)	181.6		181.6	
Zinc (g)	30		30	
Vitamin (IU)	1,500,000		7,500,000	
Vitamin D (IU)	1,700,000		1,700,000	
Vitamin E (IU)	10,000		10,000	
Biotin (mg)	100		100	
Choline (mg)	388,000		417,000	
Niacin (mg)	46,000		48,000	
Riboflavin (mg)	5,000		5,000	
Vitamin B-12	6		6	
<b>Total</b>	<b>2000</b>	<b>909.3</b>	<b>2000</b>	<b>909.3</b>

EXAMPLE 4I

## DRY DOG FOOD FORMULA

5	Ingredients	Formula 1, %	Formula 2, %
	Meat and bone meal, 50% CP	8.0	15.0
	Fish meal, 60% CP, low fat	5.0	3.0
	Soybean meal, 44% CP	12.0	-
	Soybean meal, 50% CP	-	19.0
10	Wheat germ meal, 25% CP	8.0	5.0
	Skimmed milk, dried	4.0	2.75
	Cereal grains, mixed	51.23	-
	Corn, flaked	-	23.25
	Wheat bran	4.0	-
15	Wheat, flaked	-	23.35
	Animal fat	1.75	2.75
	CLA-ester	.25	.25
	Steamed bone meal	2.0	-
	Brewers yeast	2.0	5.0
20	Fermentation solubles, dehydrated	1.0	-
	Salt and trace minerals	0.5	0.5
	Vitamin mixture	0.25	0.25
	Ferric oxide	0.02	-
25	<b>Total</b>	<b>100.00</b>	<b>100.00</b>

EXAMPLE 4J

## SEMI-MOIST DOG FOOD FORMULAS

	Ingredients	Formula 1, %	Formula 2, %
5	Soy flakes	30.9	33.5
	Meat byproducts, 70% moisture	32.0	-
	Meat and bone meal, dehydrated	-	7.3
	Water	-	25.6
10	Sugar	21.0	21.0
	Calcium and phosphorous supplement	3.3	-
	Soybean hulls	3.1	3.1
	Skimmed milk, dried	2.5	-
	Propylene glycol	2.1	2.1
15	Sorbitol	2.0	2.0
	Animal fat	.75	3.95
	CLA-ester	.25	.25
	Emulsifiers	0.9	-
	Potassium sorbate	0.35	0.35
20	Salt	0.6	0.6
	Vitamins	0.25	0.25
	Total	100.000	100.000

## CLAIMS

What is claimed is:

1. An animal feed compounded from conventional ingredients in a ration typical for the species and age of an animal, together with conjugated linoleic acid alkyl esters in a biologically active concentration.
2. The animal feed of claim 1 wherein the concentration of conjugated linoleic acid alkyl esters in said feed is about 0.05 to 3.5 percent by weight.
3. The animal feed of claim 1 wherein said conjugated linoleic acid alkyl ester is comprised of at least 50 percent up to about 99 percent by weight of octadecadienoic acid alkyl ester isomers selected from the group consisting of c9,t11-octadecadienoic acid alkyl ester and t10,c12-octadecadienoic acid alkyl ester, with less than 5 percent of an octadecadienoic acid alkyl ester selected from 11,13-octadecadienoic acid alkyl ester and trans-trans octadecadienoic acid alkyl ester, or a combination thereof.
4. A conjugated linoleic acid alkyl ester for safe use as a feed, food ingredient, or food supplement obtained by direct isomerization of an unrefined linoleic acid comprising
  - a conjugated linoleic acid alkyl ester composition of isomers in one part comprising at least 50 percent by weight of ester isomers selected from the group consisting of c9,t11-octadecadienoic acid alkyl ester and t10,c12-octadecadienoic acid alkyl ester, and combinations thereof, and
  - in a second part comprising less than 5 percent by aggregate weight of ester isomers selected from the group consisting of 8,10-octadecadienoic acid alkyl esters, 11,13-octadecanoic acid alkyl esters, and trans,trans-octadecadienoic acid alkyl esters, and
  - in a third part comprising in the range of 0.1 to 0.5 percent phosphatidyl residue remaining after isomerization of said unrefined linoleic acid.

5. The ester of claim 4 wherein said c9,t11-octadecadienoic acid alkyl ester contained in said first composition part constitutes greater than 60 percent of the total isomers of octadecadienoic acid alkyl esters.
6. The ester of claim 4 wherein said t10,c12-octadecadienoic acid alkyl ester contained in said first composition part constitutes greater than 60 percent of the total isomers of octadecadienoic acid alkyl esters.
7. A conjugated linoleic acid alkyl ester for use in domestic animal feed, food ingredients, or human dietary supplements made by the process comprising  
providing an unrefined linoleic acid alkyl ester having phosphatidyl residue in the range of about 0.1 to about 0.5 percent  
treating with an alkali alcoholate at low temperature in the presence of a monohydric low molecular weight alcohol to cause isomerization of at least 50 percent of the linoleic acid alkyl ester to conjugated linoleic alkyl ester at low temperature,  
acidifying by addition of an aqueous acid, and  
separating the linoleic conjugated linoleic acid alkyl ester from said aqueous acid without distillation.
8. The ester of claims 1-7 wherein said alkyl ester has an alkyl radical selected from the group consisting of methyl-, ethyl-, propyl-, isopropyl-, butyl-, and isobutyl-.

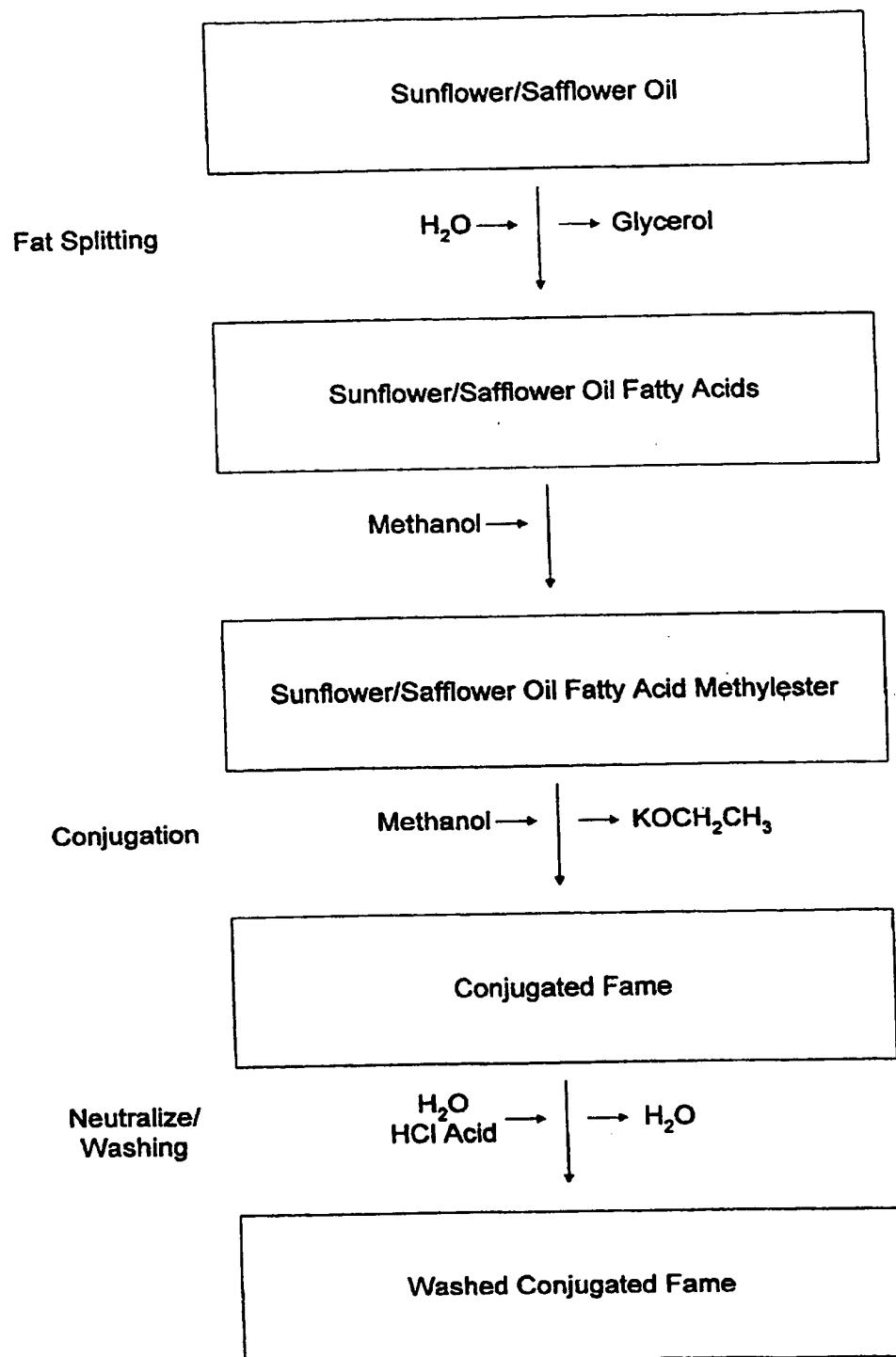


FIG. 1

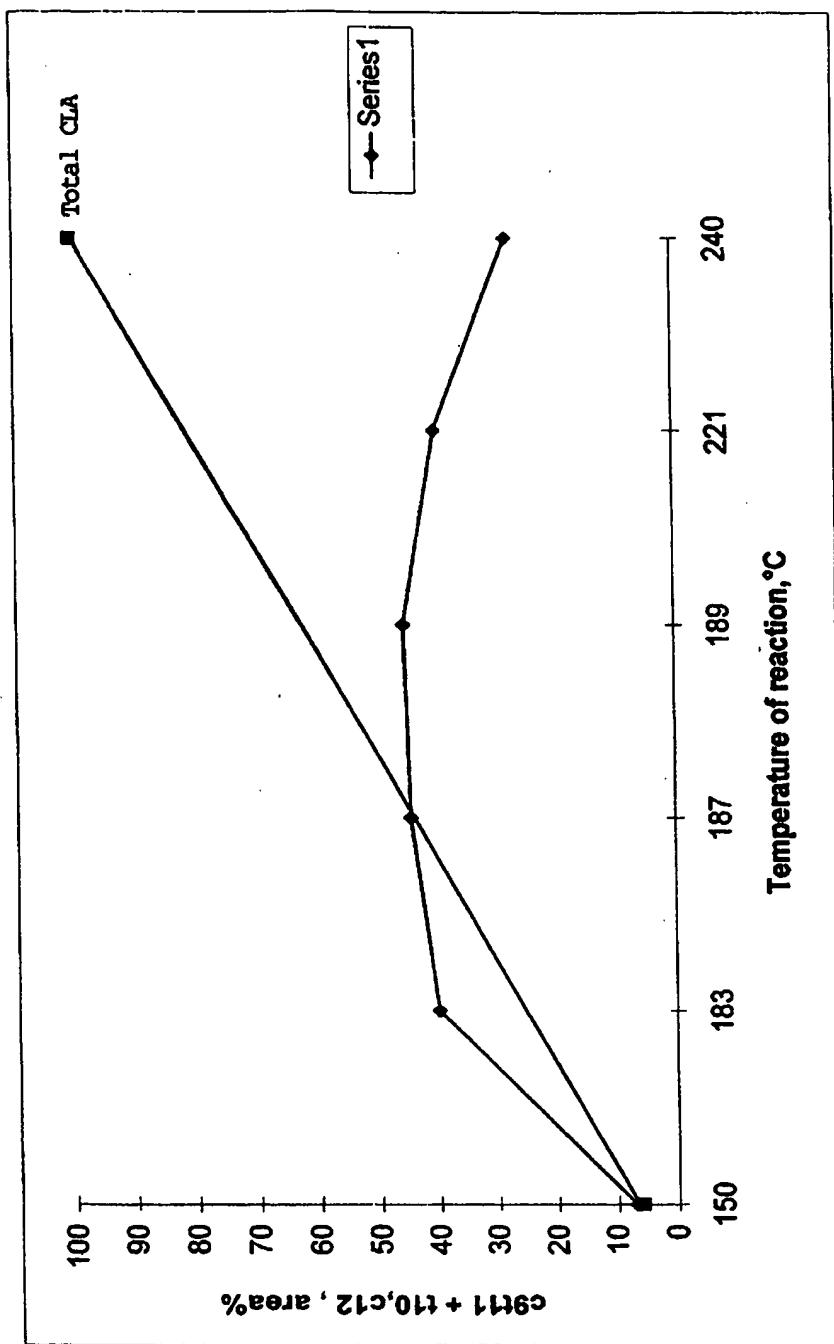


FIG. 2

VOO D. 91001194 1298

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/18094

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 47/00

US CL : 424/439; 514/558

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/439; 514/558

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
STN

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,554646 A (COOK et al) 10 September 1996, see examples 1-4.	1-8
Y	US 5,428,072 A (COOK et al) 27 June 1995, col. 3 lines 60 - col. 4 line 9, and col. 4 line 48- col. 5 line 2.	1-8

 Further documents are listed in the continuation of Box C. See patent family annex.

• Special categories of cited documents:	
•A• document defining the general state of the art which is not considered to be of particular relevance	"T"
•B• earlier document published on or after the international filing date	"X"
•L• document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"
•O• document referring to an oral disclosure, use, exhibition or other means	
•P• document published prior to the international filing date but later than the priority date claimed	"A"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

document member of the same patent family

Date of the actual completion of the international search  
05 NOVEMBER 1999Date of mailing of the international search report  
07 DEC 1999Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231  
Facsimile No. (703) 305-3230Authorized officer  
DIEDRA FAULKNER

Telephone No. (703) 308-1235